Genetic manipulation of SnRK1 for abiotic stress tolerance in wheat

Synopsis
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Introduction:

The human population is rapidly increasing and their needs for the food is also increasing thus, the agriculture productivity must increase at least by 70% as an additional 2.3 billion people will be included by 2050 (Wani et al., 2016). Various biotic and abiotic stresses, such as heat (high temperature), drought, salinity, deficiency of mineral, waterlogging and irradiance; affects crop productivity (Ashraf 2014). These factors change the plant metabolism and thus impair growth, development and crop yield (Rodriguez et al., 2019).The wheat (*Triticum aestivum L.*) 2n=6x =42 AABBDD is the common annual cereal crops and considered as major source of food grain .(Abe et al., 2019; Cui et al., 2018).

The wheat grain consists of three part, bran, endosperm and embryo, the major component of the dry weight of wheat grain is starch with 60% to 75% followed by proteins (10 – 18%) and lipid 1.5% (Ashraf 2014). Vitamins, phenolics, terpenoids and minerals are also found in different ratio in the wheat grain (Shewry et al., 2013). It is a source of fiber, vitamins B, and some trace minerals such as zinc magnesium, and iron (Aune, D et al., 2016). Therefore, wheat play an important role for both national and global food security (Zaveri & Lobell 2019). India is the second largest producer of wheat wherein Punjab alone grew 3.3 million hectare of wheat with production of 160.07 lakh tones during 2015 -2016 (Zaveri & Lobell 2019; Kaur & Dhaliwal 2018). China is the largest country for wheat production and consumption, annual production about 100 million tons of wheat yearly (Shi & Ling 2018). Worldwide the wheat grown in the high latitude semiarid regions of central and eastern Asia , Canada , USA and Kazakhstan, as well as in semiarid and hot region in North Africa , Argentina , Mexico ,Australia , south Africa and Mediterranean countries (Tricker et al., 2018).

The changes in climate worldwide affects the wheat growth and production, however wheat is able to adapt to some of the local condition and improving tolerance to biotic and abiotic stresses at some extent (Zhao et al., 2019). These adoptive mechanisms are highly expensive as it consume lot of carbohydrates such as sucrose, through activating SnRK1 gene (sucrose non –fermenting related protein kinase 1) (Campbell et al., 2000). SnRK1 gene codes heterotrimeric complex part of family protein kinases.it regulate the cell energy metabolism under energy limiting condition. (Nunes et al., 2013; Maya-Bernal et al., 2017). SnRK1 complex consists of three subunits, α subunit (catalytic subunit), β and γ subunits (regulator subunit) (Coello, P et al., 2014). The SnRK1is playing an important role in restoring energy homeostasis by regulating metabolic processes, through phosphorylation of key enzymes, as well as regulating expression of gene expression (Martínez-Barajas & Coello 2019). It regulates carbon metabolism by directly phosphorylating and inactivating the three key enzymes viz 3-hydroxy-3-methyl glutaryl CoA reductase (HMGCoA), nitrate reductase (NR) and sucrose phosphate synthase (SPS) (Wang et al., 2012). In the recent years the environmental condition changes, and these changes effect the plant growth and development, there are several study focus on this direction such as study the effect of biotic and abiotic stress to the wheat productivity, quality (Wani et al., 2016). And the role of expression SnRK1 gene under these stress by the ability of this gene to balance the energy (Martínez-Barajas & Coello 2019). This research will be focus on understand the role of SnKR1 gene under abiotic stress and tolerance in wheat.

Literature review:

Wheat and its nutritional value

Wheat *(Triticum aestivum L)* is an allohexaploid species and the most important source of food since the end of the Paleolithic age (Balfourier et al., 2019; Bird & Regina 2018).It has been

evaluated from several hundred thousand ago, *Triticum urartu* was the wild species with (2n=2x =14; AA) which is related to *Argilops speltoides* (2n=14; SS), those two species hybridized to form the allotetraploid *Triticum turgidum* (2n=4x=28 AABB) an ancestor of wild emmer wheat(Abdelrahman et al., 2020).The *Triticum turgidum* hybridized with *Aegilops tauschii* (DD) about 10000 years to form the ancestral allohexaploid *Triticum aestivum* (2n=6x=42 AABBDD)(Abdelrahman et al., 2020; Shewry et al., 2013).

The wheat grain composed of endosperm, germ and bran and contains carbohydrate components such as dietary fiber (Starch), Inulin, β-glucan and non-carbohydrate components such as carotenoids, phenolic acid, lipids (linoleic acid, palmitic acid and oleic acid) and vitamin E as well as proteins such as gliadin, glutenin, albumins and globulins (Slavin 2010; Borneo & León 2012; Katyal et al., 2017; Ashraf 2014; Schaffarczyk et al., 2014). Wheat consider as one of major staple foods consumed around the world, in view of the nutrient value, it can reduce the risk of coronary heart disease, type two diabetes, obesity also reduce risk of colorectal cancer and metabolic disorders (Deroover et al., 2019; Jensen et al.,2004; Aune et al., 2016).

Agriculture of wheat worldwide

The first farmers have spread the bread wheat germplasm from Fertile Crescent to both eastward to Asia and westward to Europe from 8500 to 2300 years ago (Balfourier et al., 2019). World wheat production was 734.2 and 760.3 million tons in 2015 and 2016 respectively (Cendoya et al., 2018). Australia, Argentina, Kazakhstan, Canada, Russian Federation, Ukraine, United States and EU countries are the most wheat exporter countries (Cendoya et al., 2018). The most important wheat producing and consuming regions in the world in 2018/2019 shown in table (1) that includes India (Sales & Exports 2018). India is one of the top producer and consumer of wheat.

The major states for wheat production in India are Punjab, Uttar, Madhya Pradesh, Bihar, Maharashtra, Haryana and Rajasthan, (Zaveri & Lobell 2019). China produce approximately 17% of global wheat production, first wheat producer in world, as it increased wheat production from 99.6 million tons in 2000 to 130.2 million tons in 2018 (Li et.al, 2019). The largest wheat production in Africa are Ethiopia and Nigeria followed by Egypt (Abdelmageed et al., 2019).

Table: 1

Sr. no.	Country	Production in Thousand metric Tons	Consumption in Thousand metric Tons
1.	European union	137.219	123.100
2.	China	131.430	152.000
3.	India	99.700	95.000
4.	Russia	71.685	40.500
5.	United state	51.287	29.162

Effect of abiotic stresses on wheat

Global climate changes like increase in temperature due to the emission of greenhouse like CO_2 and tropospheric ozone concentration (O_3) affect crop production (Zarei et al., 2019; Tao et al., 2017; Lv, Zet al., 2013). The high temperature has negative effects on quality and productivity of wheat crop (Wang et al., 2018).It leads to decrease in leaf chlorophyll damage thylakoid membrane followed by break down of PS2, lipid peroxidation of chloroplast membranes, formation of high level of reactive oxygen species. And affecting photosynthesis and grain filling (Pradhan & Prasad 2015; Yordanov 1995). Photosynthesis is sensitive and inhibited by moderate heat stress. Heat stress affect Rubisco activity that leading to inhibition of photosynthesis and productivity. (Parry et al., 2011). High temperature affect the pre-

flowering and anthesis stages more than post- flowering stage reduce grain numbers per spike (Ni et al., 2018). The increased heat stress during grain filling stage of wheat causes reduction in grain weight and therefore decrease in the yield (Rangan et al., 2020). The photosynthesis in C4 crop is happened in the mosophyll cells and bandle sheath. The gaseous CO2 initially fixed by PEPC into C4 acid and transported to bundle sheath cells for decarbxylation, releasing CO2 which is recaptured by Rubisco (Parry, M. A et al., 2011).The photorespiration rate in C4 crop are slow due to the suppression of oxygenase activity of Rubisco. It enhances the photosynthesis rate (CARMO- SILVA et al., 2008).However C3 crops plants the carbon assimilation accrues in the chloroplast stroma in mesophyll cells alone. , O2 and CO2 competes for the active sites of Rubisco ,resulting into the less about 50% of the CO2 fixation due to photorespiration (Häusler et al., 2002).The Wheat responds to climate change through various mechanism ,that includes manipulation of gene expression. It leads to biochemical, physiological and molecular changes conferring heat tolerance (Budak et al., 2013; Eid 2019). Wheat adapt to heat stress by genetic improvement of various genes and become heat tolerant plant (Cossani & Reynolds 2012). Salt and drought are abiotic stress cause crop productivity reduction, by affecting the physiological aspects of plant metabolism, creating tension (hyper -ionic and hyper osmotic) and eventually plant death. (Eid 2019).

SnRK1 gene

Sucrose Non Fermenting Related Kinase 1 (SnRK1) is a part of protein kinase family which is evolutionaroly conserved in eukaryotic organisms including yeast (SNF2), animal (AMPK) and plant (SnRK1) (Rodriguez et al., 2019). It is a heterotrimeric complex consist of α, β and γ subunits. α subunit is known as a catalytic subunit while β is a regulatory subunit and γ is nucleotide binding subunit. The α subunit exhibits on N- terminal Ser/The kinase domain. That is linked to C- terminal regulatory domain. The α subunit is activated by phosphorylation

(Crepin & Rolland 2019; Rodriguez et al., 2019). The β subunit exhibits a variable N- terminal domain undergoing myristoylation, that facilitate interaction with α and γ subunits. The γ subunit binds to ATP and thus provide energy to the complex (Crepin & Rolland 2019).

Protein kinases are catalytic enzymes plays important role in regulation of plant development by transfer of phosphate groups from one molecule to another .In plant SnRK1 respond to stress-induced alterations in AMP\ATP ratios via phosphorylation of many proteins (Campbell et al., 2000).

SnRK1 is critical in growth regulation and development of plant through a regulatory role in carbohydrate and energy metabolism (Pan 2020). SnRK1 overexpress in response to stress conditions both biotic and abiotic stress (Maya-Bernal et al., 2017). It SnRK1 regulates different processes such as photosynthesis and starch synthesis, in response to abiotic stress by modification of enzyme activity, it was found to inhibit the expression of APL3 which is involved in starch synthesis (Broeckx et al., 2016). Thus in present proposal we have planned a detailed study to understand impact of SnRK1 in heat tolerance in wheat. The study is aimed at following objectives.

Objectives of Research:

1. Silencing SnRK1 in wheat using RNAi
2. Understanding tissue specific expression of SnRK1
3. Understanding impact of SnRK1 silencing under normal and stress condition.

Importance of study:

This is the first study aimed at understanding impact of SnRK1 in growth and development of wheat. It will provide the information about the role of SnRK1 in abiotic stress tolerance. If proved our study will provide a novel candidate gene for developing abiotic stress tolerance in wheat.

Material and Methods:

- **Wheat germplasm (Bobwhite).**

The wheat germplasm, ***Triticum aestivum L***, (Bobwhite) of this research is collected from the laboratory of Dr . G. B. Zore

- **Silencing the SnRK1 by using RNAi**

1- Agrobacterium mediated wheat transformation using Gatway cloning method and PHG8 vector.

2- Develop transgenic wheat lines.

- **Determine tissues specific expression**

Expression analysis of SnRK1 gene in different tissues will be carried out

- **Impact of SnRK1 silencing under normal and stress condition.**

Impact of SnRK1 silening will be studied by growing transgenic lines under stress conditions and compared with the controls .

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